ITEM 3. INTRODUCTORY STATEMENT AND GENERAL INVESTIGATIONAL PLAN

INTRODUCTION

This Investigational New Drug Application (IND) is being submitted to conduct clinical trials with *Recombinant Adenovirus Vector Encoding the Gene for the MART-1 Melanoma Antigen (Genzyme) - Vaccine plus Interleukin-2 (Chiron).* The initial clinical study will evaluate the toxicity, immunologic reactivity, and potential therapeutic efficacy of immunization with recombinant adenovirus expressing the MART-1 melanoma antigen.

DRUG DESCRIPTION

Name:
Recombinant Adenovirus Vector Encoding the Gene for MART-1 Melanoma Antigen

Chemical structure:
The product is an adenovirus vector encoding the gene for MART-l melanoma antigen (see schematic under Item 7c).

Pharmacologic Class:
Vaccine

Bulk Drug Substance:
The Ad2/MART-1 was manufactured by Genzyme, Inc., Cambridge, MA.

Final Vaccine Drug Product:
The final vaccine drug product will be diluted and prepared at NIH by the principal investigator, his assistant, or by a pharmacist.

Formulation:
The stock solution of adenovirus vector encoding the gene for MART-1 melanoma antigen is supplied as 2.75 X 10^{11} IU per mL in both 100 μL and 250 μl aliquot. The solution will be prepared and labeled in single use crimp vials and stored at -80°C. The adenovirus vector encoding the gene for MART-1 melanoma antigen will be supplied in a 3% sucrose, PBS solution. The stock solutions will be diluted in a laminar air flow hood and administered at the following concentrations for the 10^7, 10^8, and 10^9 IU.

PREVIOUS HUMAN EXPERIENCE

Patients have been immunized with synthetic MART-1 peptide mixed with Montanide ISA under IND 5909. A brief summary of the clinical experience under IND 5909 is enclosed under Item 9. However, to the best of our knowledge no patient has been administered adenovirus encoding the gene for the MART-1 protein. Since most melanoma tumors express this protein, patients with melanoma tumors are most likely exposed to the MART-1 protein. In addition, the MART-1 protein is thought to be processed to the nonapeptide and presented on antigen presenting cells in patients with metastatic melanoma. Furthermore, *ex vivo* activated and cultured tumor infiltrating lymphocytes (TIL) containing CTLs specific for the MART-1 epitope have been administered to many patients.

The adenovirus strain and construct used for the MART-1 vaccine is identical to Genzyme's adenovirus Cystic Fibrosis (CF) vector. The adenovirus CF vector has been used in many patients. The only adverse effect of treatment with the vector has been localized inflammation.

RATIONALE

Introduction

Background

The five-year survival rate of patients with metastatic melanoma is less than 2% in most reported series. Combination chemotherapy can induce objective regression of melanoma but this treatment is rarely, if ever, curative. Tumor infiltrating lymphocytes (TIL) have been identified in patients with melanoma that appear to recognize unique cancer antigens in a MHC restricted fashion (1-3). The adoptive transfer of TIL can mediate regression in 35 to 40% of patients with advanced melanoma and studies are underway to attempt to generate more potent TIL by genetic modification of TIL or by immunization with preparations containing tumor associated antigens (1,4).

A large number of studies in experimental animals have demonstrated that the cellular rather than the humoral arm of the immune response plays the major role in the elimination of murine tumors (5). Much of this evidence was derived from studies in which the adoptive transfer of T lymphocytes from immune animals could transfer resistance to tumor challenge or in some experiments the actual elimination of established cancer. Thus, most strategies for the immunization of patients with cancer have been directed at stimulating strong T cell immune reactions against tumor associated antigens.

Most attempts at active immunization against cancer antigens have involved whole tumor cells or tumor cell fragments. However, it would be desirable to immunize specifically against unique tumor antigens that distinguish malignant from normal cells. The molecular nature of the tumor antigens recognized by T lymphocytes is poorly understood. In contrast to antibodies that recognize epitopes on intact proteins, T cells recognize short peptide fragments (8-18 amino acids) that are presented on surface class I or II major histocompatibility (MHC) molecules. It is likely that tumor antigens are presented and recognized by T cells in this fashion.

MART-1 MELANOMA ANTIGEN

In recent studies, the Surgery Branch, NCI, has identified genes that encode melanoma tumor antigens recognized by TIL in the context of the HLA-A2 class I molecule (6-8). These antigens appear to be the clinically relevant antigens responsible for mediating tumor regression in patients with advanced melanoma since the TIL used to identify these antigens were capable of mediating *in vivo* tumor regression. An antigen, which appears to be present in virtually all fresh and cultured melanomas, has been called MART-1 (Melanoma Antigen Recognized by T Cells - 1). The gene encoding this antigen has been cloned and sequenced. The MART-1 gene encodes an 118 amino acid protein of 13 kd. With the exception of melanocytes and retina, no normal tissues express this antigen and no expression of these gene products has been seen on cancers other than melanoma. Therefore, this antigen appears to represent a melanocyte lineage antigen.

The expression of the MART-1 antigen in melanomas has been demonstrated by the Surgery Branch, NCI. The MART-1 antigen was expressed on all nine tissue culture lines tested that were established from melanomas. Studies by others showed that the MART-1 antigen was expressed on 26 of 26 fresh melanomas (9). Of 14 separate TIL cells that were raised in the Surgery Branch, NCI from different HLA-$A2^{+}$ individuals, 13 of 14 recognized the MART-1 antigen. Since TIL cells that recognize this determinant have been shown to be capable of mediating cancer regression *in vivo,* it appears that the antigen is involved in cancer regression. In the present protocol, therefore, we propose to immunize patients against the MART-1 antigen in an effort to boost cellular immune responses against their cancers. For these immunizations, a recombinant adenovirus vector containing the gene encoding the MART-1 melanoma antigen was constructed.

Recombinant Adenoviruses

Adenoviruses are double stranded DNA viruses containing approximately 30-40 (kb) kilobase pairs. Over 100 different serotypes have been described, including 47 of human origin. Human adenovirus of serotypes 2 and 5 have been extensively characterized and utilized in molecular biology studies. The adenovirus genome can

be readily manipulated by recombinant DNA techniques and inserts of foreign genes are expressed.

The maximum amount of DNA that can be packaged into a virion is approximately 105% of the wild type genome. Thus, incorporation of large DNA fragments requires the deletion of wild-type viral DNA sequences. Most commonly, the DNA sequences from the E1 or E3 regions are deleted. Deletion of the E1 region produces a virus incapable of viral replication except in specifically constructed human cell lines that can provide the products of this genomic region. Thus, elimination of the E1 region provides room for the insertion of recombinant genes and also produces a virus incapable of replicating in human cells.

Human adenoviruses are ubiquitously distributed in humans and are major causative agents of acute respiratory disease. Vaccines consisting of unattenuated adenovirus in enteric capsules have been licensed for oral administration since 1980 and have been administered to millions of military recruits (13). The safety, immunogenicity and effectiveness of these vaccines have been well established and have made adenoviruses an attractive candidate for use in the development of recombinant vaccines.

Vaccines based on recombinant adenoviruses have been shown to be effective immunizing agents in animal models. Adenoviral recombinants carrying inserts of the rabies glycoprotein or antigens from the HSV or VSV viruses have been shown to raise neutralizing antibodies in rhesus monkeys, cows, pigs, dogs, foxes, striped skunks, raccoons, and mice that can protect animals against VSV and rabies infections (13). In one study, skunks and foxes were immunized via an adenoviral vector with the rabies glycoprotein and were completely protected against subsequent subcutaneous challenges with the rabies viruses which resulted in death of 100% of the control animals. Immunization of chimpanzees with recombinant adenovirus expressing either the HIV env or gag-protease genes resulted in both humoral and cellular immune responses to these antigens (14). Although most adenoviruses have been administered in enteric coated forms, recombinant adenoviruses have also been given by subcutaneous injection. Recombinant adenovirus expressing the p24 antigen of HIV was used to immunize rhesus monkeys subcutaneously and gave rise to serum antibodies against p24 (15). Human volunteers have received recombinant adenovirus containing hepatitis B surface antigen (16). In addition, there is now experience in humans receiving gene therapy for cystic fibrosis by inhalation of recombinant adenovirus. The results provide evidence that the adenoviral vectors can be safely administered and are capable of stimulating immune responses in a variety of mammals, including humans, and nonhuman primates.

Recombinant Adenoviruses: Murine Tumor Models

In the Surgery Branch, NCI, recombinant adenoviruses have been studied to evaluate their ability to immunize against an experimental tumor antigen. Tumors transduced with the gene for beta-galactosidase (beta-gal) grew vigorously in syngeneic BALB/c mice (Protocol figure 1). Mice immunized with recombinant adenovirus carrying the beta-gal antigen generated potent cytolytic T cell responses against the antigen bearing tumor (Protocol figure 2). Of particular importance were experiments showing that immunization with recombinant adenovirus expressing the beta-gal antigen could also protect mice against subcutaneous challenge of tumor bearing the beta-gal antigen (Protocol figure 3).

Immunization with recombinant adenovirus bearing the beta-gal antigen resulted in CTL production in the spleens of mice. Following *in vitro* sensitization, a reduction of established pulmonary metastases in syngeneic BALB/c mice was observed. The concomitant administration of these cells along with interleukin-2 was necessary to see a maximum effect of the adoptively transferred cells (Protocol table 1). In a representative experiment treating day 3 lung metastases for example, control mice had greater than 500 lung metastases. A group of mice which received 2×10^6 recombinant adenovirus and IL-2 generated CTL and developed only six metastases in contrast to 344 metastases when receiving these same cells without IL-2 administration.

In summary, recent research in the Surgery Branch has: identified melanoma antigens associated with tumor regression; developed recombinant adenoviruses expressing these antigens; and demonstrated that immunization with recombinant adenoviruses expressing tumor antigens could successfully protect mice from tumor challenge and could generate CTL capable of mediating cancer regression when adoptively transferred along with IL-2. These findings form the basis of the current protocol for the treatment of patients with metastatic melanoma.

CLINICAL STUDY

Indications to Be Studied

Adenovirus vector encoding the MART-1 protein will be studied in HLA-A2.1 positive patients with metastatic melanoma who have measurable disease and a life expectancy of more than three months.

Objectives

To evaluate the toxicity, immunologic reactivity, and possible therapeutic efficacy of adenovirus expressing the MART-1 melanoma antigen administered alone or in conjunction with Interleukin-2.

Study Design

Patients with metastatic melanoma who are HLA-A2.1 positive and who have failed standard effective therapy will be eligible for this trial. Patients will receive subcutaneous injections of adenovirus containing the gene encoding the MART-1 antigen. Patients will receive two successive immunizations with the same dose of adenovirus separated by a time period of four weeks. Cohorts of three patients will be treated at the following dosage levels: 10^7 pfu, 10^8 pfu, and 10^9 pfu. If any patient achieves a grade III or IV toxicity, an additional 3 patients will be treated at that dose. If a second patient develops a grade Ill or IV toxicity, the dose will not be escalated. The prior dose will be considered the maximum tolerated dose (MTD). At the MTD, three patients will be vaccinated with adenovirus followed by IL-2 administered in each of the following groups.

1. IL-2, subcutaneous injection of 250,000 IU/kg daily for five days and repeated once after a two day rest.

2. IL-2, intravenous infusion of 72,000 IU/kg every eight hours for seven days, not to exceed 21 doses.

3. IL-2, intravenous infusion of 720,000 IU/kg every eight hours for five days, not to exceed 15 doses.

Sample Size

Approximately, 45 patients will be accrued for this phase I protocol. However, the exact number may vary depending upon observed toxicities.

Eligibility Criteria

Any patient with measurable metastatic melanoma age ≥ 18, a performance status of ECOG 0, 1 or 2, and an expected survival of greater than three months will be considered. Eligible patients must be HLA-A2.1 positive with a confirmed diagnosis by a NIH pathologist. Patients that require steroid therapy or have had prior therapy less than four weeks from the start of the study will not be eligible for study participation. In

addition, any patient with active systemic infections, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system will not be included in the study. Patients must meet the following criteria: white blood count > 3000/mm^3, platelet count > 100,000/mm^3, hemoglobin > 8.0 g/dL, serum ALT/AST < four times the upper limit of normal, serum creatinine ≤ 1.6 mg/dL, bilirubin ≤ 2.0 mg/dL, and seronegative for HIV antibody and Hepatitis B antigen. Women of child-bearing potential must have a negative pregnancy test and be willing to use an effective method of contraception during the trial.

Potential Toxicities

The MART-1 antigen has been shown to be expressed on retina and melanocytes, though at levels 10 to 100 times less than human melanoma cell lines. The potential exists that immune responses generated against melanocyte-melanoma lineage antigens such as MART-1 may react against normal cells. TILs with anti-melanocyte-melanoma reactivities have been administered to patients with advanced melanoma, and although sporadic cases of vitiligo were seen (probably related to anti-melanocyte immune reactions) no adverse ophthalmologic effects were observed. The only toxicity seen in patients given up to 10 mg of MART-1 peptide in Montanide ISA under IND 5909 has been a transient skin rash in two patients. There is the potential that patients may also react locally to the adenovirus with inflammation at the site of injection.